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(hairpin near0 probe\$) same hybridiz\$

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USPT	12 same probe\$	62	L3
USPT	11 same hybridiz\$	96	L2
USPT	(reduc\$ or decreas\$) same (background near0 signal\$)	1057	L1

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L5: Entry 1 of 1

File: USPT

Aug 21, 2001

DOCUMENT-IDENTIFIER: US 6277570 B1

TITLE: Nucleic acid sequence detection employing probes comprising non-nucleosidic coumarin derivatives as polynucleotide-crosslinking agents

BSPR:

Furthermore, oligonucleotide probe-based assay methods are known to depend upon careful optimization of the wash stringency. If the wash conditions are too stringent, then probe/target hybrids will be denatured, resulting in a decrease in the amount of signal in the assay. If the wash conditions are not sufficiently stringent, then non-specifically bound probes or mismatched probe/target hybrids will remain in the assay medium, resulting in high levels of non-specific or background signal in the assay. Optimal conditions are necessarily different for each probe because hybridization is a sequence-dependent phenomenon and would also depend on the extent to which near-homologous sequences are present in the sample.

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L4: Entry 9 of 9

File: USPT

Jul 28, 1987

DOCUMENT-IDENTIFIER: US 4683194 A

TITLE: Method for detection of polymorphic restriction sites and nucleic acid sequences

DEPR:

As discussed above, if there are sufficient background signals, after hybridization an unlabeled oligonucleotide (blocking oligomer) may be added to the reaction mixture which is specific for the probe utilized. This oligomer is complementary to the probe but has at least one base pair mismatch within each restriction site being detected, so that the labeled probe not already hybridized to the nucleic acid will hybridize to the blocking oligomer and therefore will not be cleaved by digestion with the restriction enzyme(s) recognizing each restriction site being detected. The function of the blocking oligomer is to eliminate the non-specific hybridization of any excess labeled probe to the non-polymorphic nucleic acid fragments under the conditions of reduced stringency necessary for the action of the subsequently added restriction enzyme. The number of base pair mismatches within each restriction site will depend mainly on the position of the restriction site within the probe.

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L5: Entry 1 of 11

File: USPT

Jul 17, 2001

DOCUMENT-IDENTIFIER: US 6261784 B1

TITLE: Detection of nucleic acids by strand displacement

BSPR:

Alternatively, the donor and acceptor may be linked to a single oligonucleotide such that there is a detectable difference in the fluorescence properties of one or both when the oligonucleotide is unhybridized vs. when it is hybridized to its complementary sequence. In this format, donor fluorescence is typically increased and energy transfer/quenching are decreased when the oligonucleotide is hybridized. For example, a self-complementary oligonucleotide labeled at each end may form a hairpin which brings the two fluorophores (i.e., the 5' and 3' ends) into close proximity where energy transfer and quenching can occur. Hybridization of the self-complementary oligonucleotide to its complement on a second oligonucleotide disrupts the hairpin and increases the distance between the two dyes, thus reducing quenching. A disadvantage of the hairpin structure is that it is very stable and conversion to the unquenched, hybridized form is often slow and only moderately favored, resulting in generally poor performance. Tyagi and Kramer (1996, Nature Biotech. 14, 303-308) describe a hairpin labeled as described above with a detector sequence in the loop between the self-complementary arms of the hairpin which form the stem. The base-paired stem must melt in order for the detector sequence to hybridize to the target and cause a reduction in quenching. A "double hairpin" probe and methods of using it are described by B. Bagwell, et al. (1994, Nucl. Acids Res. 22, 2424-2425; U.S. Pat. No. 5,607,834). These structures contain the target binding sequence within the hairpin and therefore involve competitive hybridization between the target and the self-complementary sequences of the hairpin. Bagwell solves the problem of unfavorable hybridization kinetics by destabilizing the hairpin with mismatches, thus favoring hybridization to the target.

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L6: Entry 1 of 1

File: USPT

Jun 1, 1993

DOCUMENT-IDENTIFIER: US 5215899 A

TITLE: Nucleic acid amplification employing ligatable hairpin probe and transcription

ABPL:

Specific nucleic acid sequences are amplified through the use of a hairpin probe which, upon hybridization with and ligation to, a target sequence is capable of being transcribed. The probe comprises a single stranded self-complementary sequence which, under hybridizing conditions, forms a hairpin structure having a functional promoter region, and further comprises a single stranded probe sequence extending from the 3' end of the hairpin sequence. Upon hybridization with a target sequence complementary to the probe sequence and ligation of the 3' end of the hybridized target sequence to the 5' end of the hairpin probe, the target sequence is rendered transcribable in the presence of a suitable RNA polymerase and appropriate ribonucleoside triphosphate (rNTPs). Amplification is accomplished by hybridizing the desired target nucleic acid sequence with the probe, ligating the target sequence to the probe, adding the RNA polymerase and rNTPs to the separated hybrids, and allowing transcription to proceed until a desired amount of RNA transcription product has accumulated. The amplification method is particularly useful in assays for the detection of particular nucleic acid sequences.

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L4: Entry 3 of 9

File: USPT

Jul 10, 2001

DOCUMENT-IDENTIFIER: US 6258536 B1

TITLE: Expression monitoring of downstream genes in the BRCA1 pathway

DEPR:

In a preferred embodiment, background signal is reduced by the use of a detergent (e.g., C-TAB) or a blocking reagent (e.g., sperm DNA, cot-1 DNA, etc.) during the hybridization to reduce non-specific binding. In a particularly preferred embodiment, the hybridization is performed in the presence of about 0.5 mg/ml DNA (e.g., herring sperm DNA). The use of blocking agents in hybridization is well known to those of skill in the art (see, e.g., Chapter 8 in P. Tijssen, supra.) The stability of duplexes formed between RNAs or DNAs are generally in the order of RNA:RNA>RNA:DNA>DNA:DNA, in solution. Long probes have better duplex stability with a target, but poorer mismatch discrimination than shorter probes (mismatch discrimination refers to the measured hybridization signal ratio between a perfect match probe and a single base mismatch probe). Shorter probes (e.g., 8-mers) discriminate mismatches very well, but the overall duplex stability is low.